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Southernmost records of Entomophthoromycotina. Updated review of Entomophthoralean fungal insect pathogens of Argentina

Registros más australes de Entomophthoromycotina. Revisión de los hongos Entomophthorales patógenos de insectos de la Argentina

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ABSTRACT

The objective of this work is to further update the records of Argentinean Entomophthoralean fungal species and their geographical distribution. Our survey includes five new records of *Zoophthora radicans* infecting insect hosts belonging to the orders Diptera, Hemiptera, and Lepidoptera from Pampasic and Littoral regions of Argentina. These new records not only increase the host range of Entomophthoralean fungi but also their geographical distribution around the world.

Keywords. Diptera, entomopathogenic fungi, Hemiptera, Lepidoptera, South America

RESUMEN

El objetivo del presente trabajo es actualizar el conocimiento de especies de hongos Entomophthorales de la Argentina y su distribución geográfica. Como resultado incluimos cinco nuevos registros de *Zoophthora radicans* infectando insectos de los órdenes Diptera, Hemiptera y Lepidoptera de las regiones pampásica y litoral de la Argentina. Estas nuevas citas no solo incrementan el espectro hospedador de los hongos Entomophthorales sino también su distribución geográfica a nivel mundial.

Palabras clave. Diptera, Hemiptera, hongos entomopatógenicos, Lepidoptera, Suramérica

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INTRODUCTION

Entomophthoromycota (Phylum Zoopagomycota) is a fungal subphylum, which includes a great diversity of species that predominantly infects insects. The previous reports and articles on the subject recorded for Argentina were focused on taxonomy, biodiversity, epizootiology and some laboratory evaluations of biological activity of species *Batkoa* sp., *Conidiobolus coronatus* (Costantin) A. Batko, *C. obscurus* (I.M. Hall & P.H. Dunn) Remaud. & S. Keller, *Entomophaga grylli* (Fresenius) Batko, *E. planchoniana* Cornu, *E. ferdinandii* S. Keller, *Neozygites* sp., *N. fresenii* (Nowak.) Remaud. & S. Keller, *Pandora* sp., *P. dipterigena* (Thaxt.) Humber, *P. gammae* (Weiser) Humber, *P. neoaphidis* (Remaud. & Hennebert) Humber, *P. nouryi* (Remaud. & Hennebert) Humber, *Zoophthora* sp. and *Z. radicans* (Bref.) A. Batko.

Entomophthoralean fungi infecting insects have been studied and reported from all over the world (Eilenberg *et al.* 1987, Keller 1987, Feng *et al.* 1990, Eilenberg *et al.* 1992, Balazy 1993, Santamaría and Girbal 1996, Hatting *et al.* 1999, Nielsen *et al.* 2001, Pell *et al.* 2001, Steinkraus and Boys 2005, Jensen *et al.* 2009, Hajek *et al.* 2012, Humber 2012), while this fungal group was poorly studied in South America (Aruta *et al.* 1974, 1984, Delalibera *et al.* 1992, Alzugaray *et al.* 1999). The first Argentinean reference was published by Fresa (1979) but in the last decade, the number of publications about this fungal group has increased (Méndez-Sánchez *et al.* 2001, 2002a, b, 2009, Delalibera *et al.* 2004, López-L and Scorsetti 2007, Scorsetti and López-L 2007, Toledo *et al.* 2007, 2008a, b, Jensen *et al.* 2009, Alzugaray *et al.* 2010, Scorsetti *et al.* 2010, 2012, Sosa Gómez *et al.* 2010, Manfrino *et al.* 2013, 2014a, b, c, Montalva *et al.* 2016a, b). The overall goal of this work is to follow this endeavor and further update the knowledge of Argentinean Entomophthoralean fungal species with new records that expanded their insect host range and their geographical distribution.

MATERIALS AND METHODS

Survey of Entomophthoralean fungi were performed collecting dead and alive insects in different types of environments as poultry houses (houseflies), crops (Lepidoptera and Hemiptera) and natural preserved areas (Diptera). Insects infected with Entomophthoralean fungi were collected from under plant leaves, walls, and leaf litter in several localities from Argentina (Fig. 1) during 2004–2014.

Fungal infected insects were used for actively discharging conidia in a typical “descendent discharge” according to the methodology described by Eilenberg *et al.* (1992), Keller (2006, 2007). Conidia were cultured on Sabouraud dextrose agar with yeast extract + egg yolk + skimmed milk (Hajek *et al.* 2012) and incubated at 20 °C in darkness. Fungal structures were stained with aceto-orcein 1 %. Observations were performed by using an Optic Microscope Olympus CH30 and photographs with a differential interference contrast fitted with a Power Shot A80 camera. The taxonomic identification was based on the morphology of conidiophores, morphology, and sizes of conidia, rhizoids and cystidia and presence or absence of resting spores, using the keys and monographs of Balazy (1993), Keller (2007) and Humber (2012).

RESULTS

Entomophthoralean species recorded up to date in Argentina are listed below. This information includes a brief description of the five new records of *Zoophthora radicans* (Bref.) Batko, infecting insect hosts belonging to the orders Diptera, Hemiptera, and Lepidoptera. These new records, marked with an asterisk in the checklist, not only increase the host range of Entomophthoralean fungi but also their geographical distribution.

As a result of this study, it was possible to obtain and preserved three fungal isolates of *Z. radicans*, which were deposited in the Agricultural Research Service Entomopathogenic Fungi collection (ARSEF) (Ithaca, New York, USA) under the accession numbers ARSEF 5814, ARSEF 6917 and ARSEF 8466. The other records reported as new in this study are kept as reference dried material (*exicata*) or as permanent microscopic slides (mounted in LPAO Lactophenol-aceto-orcein and sealed with nail polish) at CEPAVE Fungarium Collection (Centro de Estudios Parasitológicos y de Vectores) (La Plata, Buenos Aires, Argentina).

Entomophthoraceae

Entomophaga grylli (Fresen.) A. Batko

Synonyms: *Conidiobolus grylli* (Fresen.) Remaud. & S. Keller,
= *Empusa grylli* (Fresen.) Nowak
= *Entomophthora grylli* Fresenius.

Material examined: ARGENTINA. BUENOS AIRES: General Villegas 35°02'00" S, 63° 01'00" W, Villa Saurí 37°7'0.12" S; 62°58'59.87" W and Benito Juárez (37°49'00" S, 57°49'00" W. LA PAMPA: Eduardo Castex 35°54'S; 64°18' W and Santa Rosa 36°37' 13"S, 64°17'26" W.



Figure 1. Distribution of Entomophthoralean sampling sites for Argentina.

Hosts: Orthoptera Acrididae, several species.

References: (Fresa 1971, Lange 1996).

***Entomophthora ferdinandii* S. Keller**

Synonyms: *Entomophthora ferdinandii* S. Keller [as 'ferdinandi']

Material examined: ARGENTINA. BUENOS AIRES: La Plata 34°05'05" S, 58°10'26" W, 23. IX. 2001. C.C. López-L (CEPhe22).

Host: *Musca domestica* L. (Diptera: Muscidae) (Fig. 2a).

References: (López-L et al. 2006, Jensen et al. 2009).

***Entomophthora planchoniana* Cornu**

Synonyms: *Empusa planchoniana* (Cornu) Thaxt.
= *Myiophyton planchonianum* (Cornu) Arx.

Material examined: ARGENTINA. BUENOS AIRES: La Plata, Colonia Urquiza 34°56'19.2" S, 58°6'3.8" W, 1. IV. 2004), SANTA FE: Rafaela and Monte Vera, 31°12'3.67" S, 61° 30'25.83" W, 24.VI.2010.

Host: Hemiptera Aphididae, several species (Figs. 2b, c).
A.C. Scorsetti, R.G. Manfrino, C.C. López-L (LPSC 47458).

References: (López-L and Scorsetti 2006, Scorsetti et al. 2007, Jensen et al. 2009, Manfrino et al. 2013, 2014 a, c).

***Pandora gammae* (J. Weiser) Humber**

Synonyms: *Entomophthora gammae* (J. Weiser) D.M. MacLeod & Müll-Kög.

= *Erynia gammae* (J. Weiser) Glare & Milner,

= *Tarichium gammae* J. Weiser

= *Zoopthora gammae* (J. Weiser) Balazy.

Material examined: ARGENTINA. BUENOS AIRES: Chivilcoy, 34°54'00" S, 60°02'00" W 1. IV. 1996, and CORDOBA: Manfredi, 31°49' S, 63°46' W.

Hosts: *Anticarsia gemmatilis* (Hübner) and *Rachiplusia nu* (Guenée) (Lepidoptera: Noctuidae), C.C. López-L. Herbarium material accession number CEP100/ENT98.

References: (Edelstein and Lecuona 2003, López-L and Scorsetti 2006).

***P. neoaphidis* (Remaud. & Hennebert) Humber**

Synonyms: *Erynia neoaphidis* Remaud. & Hennebert,
= *Zoophtora neoaphidis* (Remaud. & Hennebert)
Balazy,
= *Zoophtora neoaphidis* (Remaud. & Hennebert)
Ben Ze'ev & R.G. Kenneth.

Material examined: ARGENTINA. BUENOS AIRES: La Plata, Colonia Urquiza, 34°56'19.2" S, 58°6'3.8" W, SANTA FE: Monte Vera, 31°32'49"S; 60°41'33"W, 16. X. 2010.

Hosts: Hemiptera Aphididae, several species (Figs. 3a, b).

A.C. Scorsetti, R.G. Manfrino (LPSC 47466, LPSC47459, LPSC 47460)

References: (Scorsetti et al. 2007, 2012, Manfrino et al. 2013, 2014a, b, c)

***P. nouri* (Remaud. & Hennebert) Humber**

Synonyms: *Erynia nouri* Remaud. & Hennebert,
= *Zoophtora nouri* (Remaud. & Hennebert)
Ben Ze'ev & R.G. Kenneth.

Material examined: ARGENTINA. BUENOS AIRES: La Plata, 34°55'49" S, 57°56'32" W, 28.VIII. 2005.

Host: *Heterocaecilius* sp. (Psocodea: Pseudocaeciliidae) (Figs. 3c, d) A.V. Toledo (LPSC 47784).

Reference: (Toledo et al. 2008b).

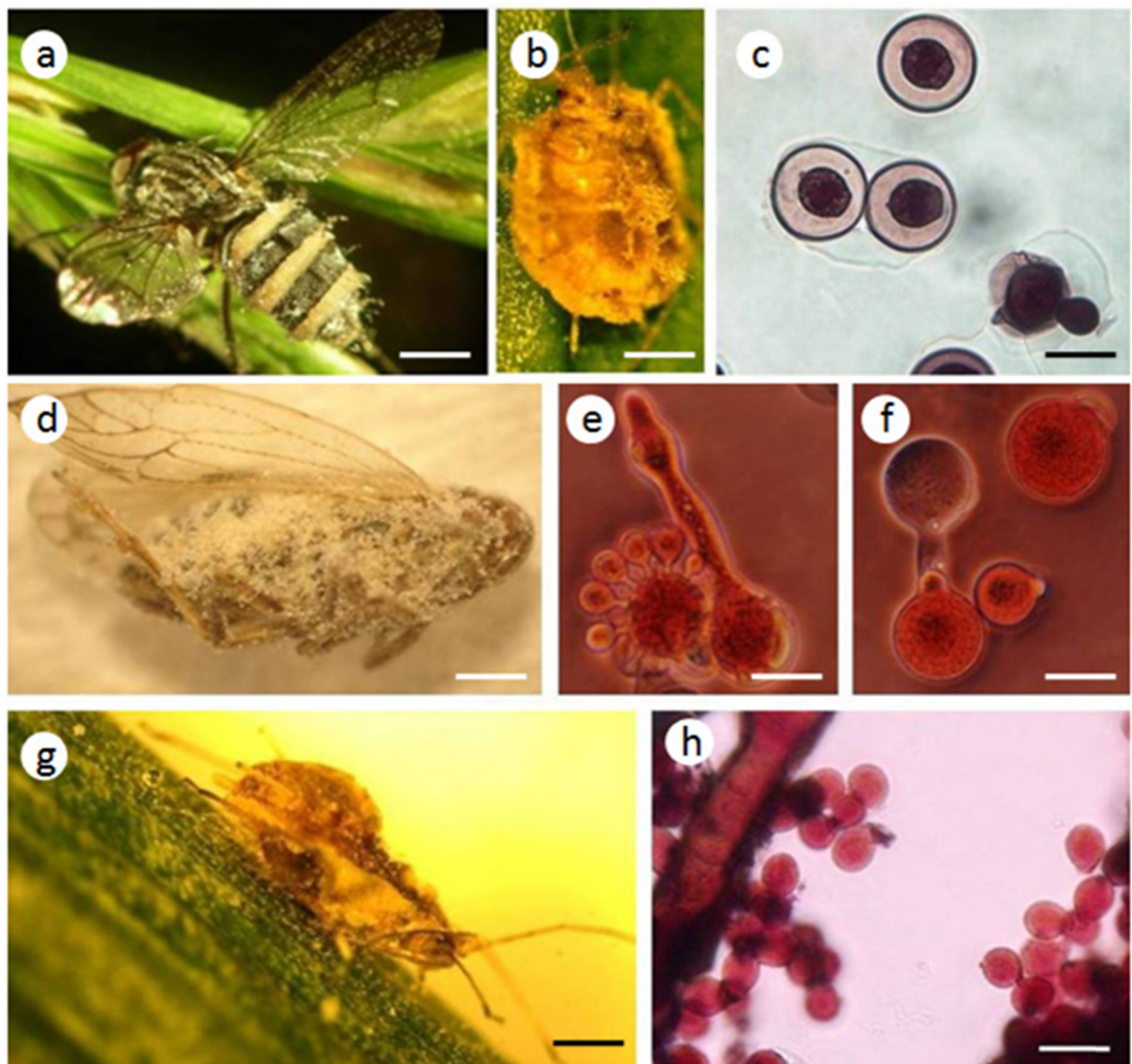


Figure 2. Insect hosts infected with Entomophthoralean fungi species and conidia. **a.** *Musca domestica* infected with *Entomophthora ferdinandii*; **b.** *Myzus persicae* infected with *E. planchoniana*; **c.** Primary conidia of *E. planchoniana*; **d.** *Delphacodes kuscheli* infected with *Conidiobolus coronatus*; **e.** Microconidia of *C. coronatus* isolated from *Ollarius dimidiatus*; **f.** Primary and secondary conidia of *C. coronatus* isolated from *O. dimidiatus*; **g.** *Schizaphis graminum* infected with *Neozygites fresenii*; **h.** Primary conidia of *N. fresenii*. Scale bars: a = 1.3 mm, b = 0.6 mm, c = 20 µm, d = 0.5 mm, e = 33.5 µm, f = 29 µm, g = 2.5 mm, h = 28.3 µm.

Zoophthora radicans (Bref.) A. Batko

Synonyms: *Empusa radicans* Brefeld.

Material examined: ARGENTINA. BUENOS AIRES: La Plata, Arana 35°0'19.8" S, 57° 55'30" W and Colonia Urquiza 34°56'19.2" S, 58°6'3.8" W, Berazategui, Parque Pereyra Iraola 34°50'36.5" S, 58°33.2" W and Magdalena, General Mansilla 35°5' 30.7" S, 57°45'20.6" W. SANTA FE: Monte Vera 31°32'58.21" S, 60°41'34.74" W, Rafaela 31°12'3.67" S, 61° 30' 25.83" W, Sarmiento 31°3'24.84" S, 61°10'13.29" W and Ceres 29°52'55.38"S, 61°56'25" W, 1.VI.2010.

Hosts: Hemiptera Aphididae, several species (Figs. 3e, f). Culture collection accession numbers ARSEF 7208, ARSEF 7455, ARSEF 7788, ARSEF 7789. A.C. Scorsetti, R.G. Manfrino, (LPSC 47467, LPSC 47460).

References: (Scorsetti et al. 2007, Manfrino et al. 2013, 2014a, b, c)

*** Z. radicans**

New record. Material examined: ARGENTINA. CORRIENTES: Las Marías (28°6'44" S, 56°3'5" W, 1. IX. 2009. S. Durman, C.C. López-L. (CEPhe42)

Description. Microscopic characters: Primary conidia clavate and uninucleate: $17.2 (\pm 0.5) \times 5.8 (\pm 0.4) \mu\text{m}$. Secondary conidia: $9.2 (\pm 0.5) \times 5.1 (\pm 0.2) \mu\text{m}$. Capilliconidia: $8.2 (\pm 1.2) \times 2.2 (\pm 0.2) \mu\text{m}$. Rhizoids and resting spores were not observed.

Host: Adults of *Gyropsilla spegazziniana* (Lizer & Trelles) (Hemiptera: Psyllidae) collected from *Ilex paraguariensis* (St.-Hil.) (Aquifoliaceae)

*** Z. radicans**

New record. Material examined: ARGENTINA. BUENOS AIRES: La Plata, Colonia Urquiza, 34°55' 43"S, 58°1' 48" W, 5. VI. 2001 C.C. López-L, C.C. (ARSEF 6917).

Description. Microscopic characters: Primary conidia clavate and uninucleate: $16.5 (\pm 0.6) \times 6.3 (\pm 0.3) \mu\text{m}$. Secondary conidia: $9.7 (\pm 0.5) \times 4.2 (\pm 0.32) \mu\text{m}$. Capilliconidia: $7.7 (\pm 1.3) \times 2.37 \mu\text{m}$.

Host: Larva of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) collected from *Solanum lycopersicum* L. (Solanaceae) in covered greenhouses.

*** Z. radicans**

New record. Material examined: ARGENTINA. BUENOS AIRES: La Plata, 34°55'30" S, 57°56'38" W, 5. V. 2007. A.V. Toledo, (ARSEF 8466).

Description. Microscopic characters: Conidiophores digitately branched. Primary conidia clavate and uninucleate with basal papilla rounded: 14.9 ± 0.2 (12–16.8) μm in length \times 5.6 ± 0.1 (4.8–7.2) μm in width. No rhizoids, cystidia or resistance spores were observed.

Host: Females of unidentified species of Diptera Chironomidae collected from walls. (Figs. 3g, i).

*** Z. radicans**

New record. Material examined: ARGENTINA. SANTA FÉ: Monte Vera 31°32'58" S, 60°41'34" W, 13.V. 2011. R.G. Manfrino (CEPhe50).

Description. Microscopic characters: Conidia uninucleate, primary conidia elongate, papilla generally conical demarcated with a slight protuberance from the body of the conidia: 20.2 ± 2.3 (15.7–24.0) in length \times 6.2 ± 0.8 (4.9–7.4) μm in width. Secondary conidia as capilliconidia: 20.0 ± 1.8 (18.9–24.4) \times 7.1 ± 0.5 (6.4–7.9) \times 46.9 ± 9.7 (35.8–60.3) μm . Thin rhizoids compound with specialized adhesive disc. No resistance spores were observed.

Host: Larvae of *Plutella xylostella* L. (Lepidoptera: Plutellidae) collected from *Brassica oleracea* var. *capitata* L. (Brassicaceae).

*** Z. radicans**

New record. Material examined: ARGENTINA. BUENOS AIRES: Los Hornos, 34°59'8" S, 57°59'53" W, 28.V. 1998. López-L. C.C. (ARSEF 5814).

Description. Microscopic characters: Primary conidia clavate and uninucleate: $15.5\text{--}22.5 \times 6.3\text{--}8.4 \mu\text{m}$. Secondary conidia as capilliconidia $17.3\text{--}21.8 (\pm 0.5) \times 4.6\text{--}5.9 (\pm 1.2) \mu\text{m}$. Resting spores not observed.

Host: Larva of *Epinotia aporema* (Walsingham) (Lepidoptera: Tortricidae) collected from *Lupinus* sp. (Fabaceae).

Ancylistaceae**Conidiobolus coronatus (Costantin) A. Batko**

Synonyms: *Boudierella coronata* Costantin

= *Conidiobolus villosus* G.W. Martin

= *Delacroixia coronata* (Costantin) Sacc. & P. Syd.

= *Entomophthora coronata* (Costantin) Kevorkian.

Material examined: ARGENTINA. BUENOS AIRES: La Plata 35°54'18" S, 57°57'0.3" W and Los Hornos 34°52'S, 57°58' W. 19. VIIII. 2003. A.V. Toledo (LPSC 47779)

Hosts: *Delphacodes kuscheli* (Fennah), *D. haywardi* Muir (Hemiptera: Delphacidae) and *Oliarus dimidiatus* Berg (Hemiptera: Cixiidae) (Figs. 2a, c).

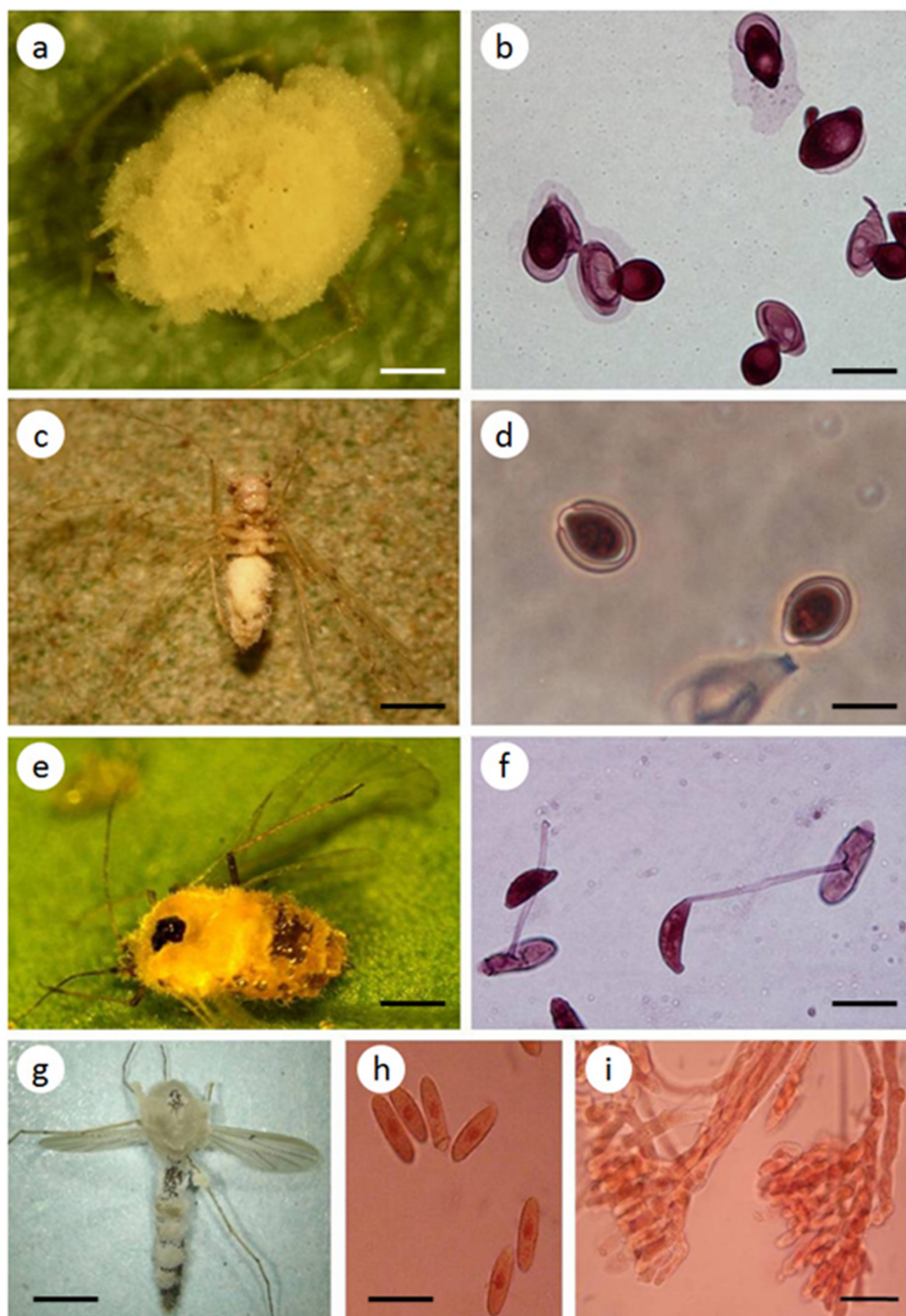


Figure 3. Different type of conidia of Entomophthoralean fungal species. **a.** *Myzus persicae* infected with *Pandora neoaphidis*; **b.** Bitunicate primary conidia of *P. neoaphidis*; **c.** *Heteroecilius* sp. infected with *P. noursy*; **d.** Bitunicate primary conidia of *P. noursy*; **e.** *Brevicoryne brassicae* infected with *Zoophthora radicans*; **f.** Capilliconidia of *Z. radicans*; **g.** Unidentified species (Diptera: Chironomidae) infected with *Z. radicans*; **h.** *Z. radicans* primary conidia, and **i** conidiophores. Scale bars: a = 0.4 mm, b = 28 μ m, c = 0.3 mm, d = 12.3 μ m, e = 0.5 mm, f = 15.3 μ m, g = 2 mm, h = 23 μ m, i = 12.5 μ m.

Culture collection accession number ARSEF 7203.

References: (Toledo et al. 2007, 2008a).

C. obscurus (I.M. Hall & P.H. Dunn) Remaud. & S. Keller

Synonyms: *Empusa thaxteriana* Petch,

= *Entomophaga obscura* (I.M. Hall & P.H. Dunn)

A. Batko,

= *Entomophaga thaxteriana* A. Batko,

= *Entomophthora ignobilis* I.M. Hall & P.H. Dunn,

= *Entomophthora obscura* I.M. Hall & P.H. Dunn,

= *Entomophthora thaxteriana* I.M. Hall & J. Bell,

= *Entomophthora thaxteriana* Petr.

Material examined: ARGENTINA. BUENOS AIRES: Bera-zategui, Parque Pereyra Iraola 34°50'36.5" S, 58°5'33.2" W, La Plata, Colonia Urquiza 34°56'19.2" S, 58°6'3.8" W and Los Cardales 34°19'25.2" S, 58°59'41.4" W 1. XI. 2002. A.C. Scorsetti (LPSC 47461).

Hosts: *Neotoxoptera formosana* (Takahashi), *Nasonovia ribisnigri* (Mosley), *Brevicoryne brassicae* L. and *Myzus* sp. (Hemiptera: Aphididae).

References: (Scorsetti et al. 2007, Comerio et al. 2008)

Neozygitaceae

***Neozygites fresenii* (Nowak.) Remaud. & S. Keller**

Synonyms: *Empusa fresenii* Nowak.

= *Entomophthora fresenii* (Nowak.) M.A. Gust.

= *Entomophthora neri* M.A. Gust.

= *Neozygites aphidis* Witlaczil,

= *Triplosporium fresenii* (Nowak.) A. Batko.

Material examined: ARGENTINA. SANTA FE: Monte Vera 31°32'58.21" S, 60°41'34.74" W and Rafaela (31°12'6.62" S, 61°30'11.14" W, 3. III.2011.

Hosts: Hemiptera Aphididae, several species (Figs. 2g, h). R.G. Manfrino (LPSC 47468).

References: (Manfrino et al. 2014a, b).

DISCUSSION

Knowledge about distribution and diversity of Entomophthoralean species infecting arthropods in Argentina is scarce and spread out.

As result of our study, the insect host range of these fungi was extended, being Hemiptera the order with the highest number of species infected with Entomophthoralean fungi, and followed by Lepidoptera and Diptera species, even though there are a lot of other insect groups that up to date there were never surveyed in order to look for Entomophthoralean fungi in Argentina yet.

Molecular taxonomic identification and characterization of fungal species are being to be submitted for publication soon, and these studies will contribute to reinforce and strongly support the present records (Manfrino, com. Pers.). We hope that as a result of future researches the number of species recorded in Argentina will increase in accordance with the diversity of habitats that owns the country.

AUTHOR'S CONTRIBUTION

RM and AT collected the samples; CL identified the specimens; CL and AT wrote the text. AG made the map and figures.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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